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Supplementary information

The role of the individual Lhcas in Photosystem I excitation energy Trapping

Emilie Wientjes, Ivo H.M van Stokkum, Herbert van Amerongen, and Roberta Croce

SI1 Absorption spectra of PSI complexes.

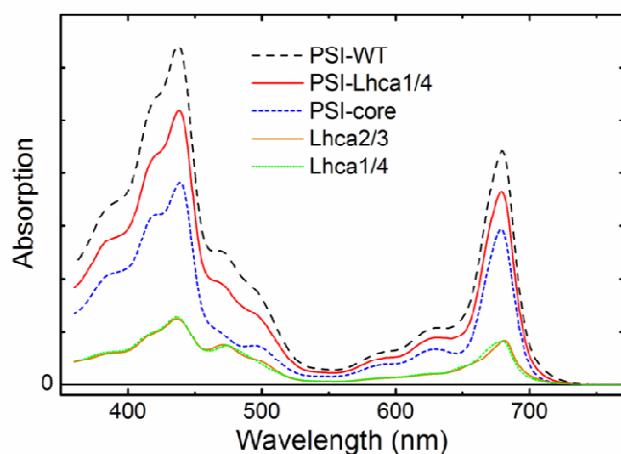


FIGURE SI1 RT absorption spectra of PSI-complexes. Spectra are normalized in the Q_y region based on 170 Chls for PSI-WT, 135 Chls for PSI-Lhca1/4, 100 Chls for PSI-core and 35 Chls (see SI. 2 A) for both Lhca1/4 and Lhca2/3. The oscillator strengths of Chl *b* is assumed to be 0.7 times that of Chl *a* in the Q_y region (630-750nm).

SI2A Fraction of PSI core excitation

In order to obtain the fraction of core and LHCI excitation at specific wavelengths a few factors have to be taken in account. First, the efficiency of energy transfer from the carotenoids (Car) to the Chls. For the Lhca1/4 and Lhca2/3 dimers we obtained these efficiencies by comparing the absorption and excitation spectra. The absorption spectra were fitted with the spectra of all pigments in a protein environment (1). The excitation spectra are fitted with the same spectral forms, allowing the carotenoids to contribute less as compared to the absorption spectra see figure SI2.1. In this way Car to Chl transfer efficiencies of 70% for Lhca1/4 and 64% for Lhca2/3 were obtained. For the PSI core a 70% Car to Chl efficiency was used (2). The absorption spectrum of PSI-LHCI needs to be described with the spectra of the core and LHCI. This was done in two ways. Method I: the core was normalized to 100 Chls *a* in the Q_y region, and each dimer to 35 Chls, which gives the spectra shown in Fig SI2.2. A disadvantage of this method is that there is a small difference between the absorption spectrum of the whole complex and the sum of its parts. Therefore, in Method 2 all absorption of the complex that is not due to the core is ascribed to LHCI, and these values are used in this study. In both methods the gap-pigments are counted as LHCI pigments, and the Car to Chl transfer efficiency is assumed to be the same as obtained for LHCI.

Table SI2.1 shows the effective excitation of the core compartment after correction for the Car to Chl transfer efficiency.

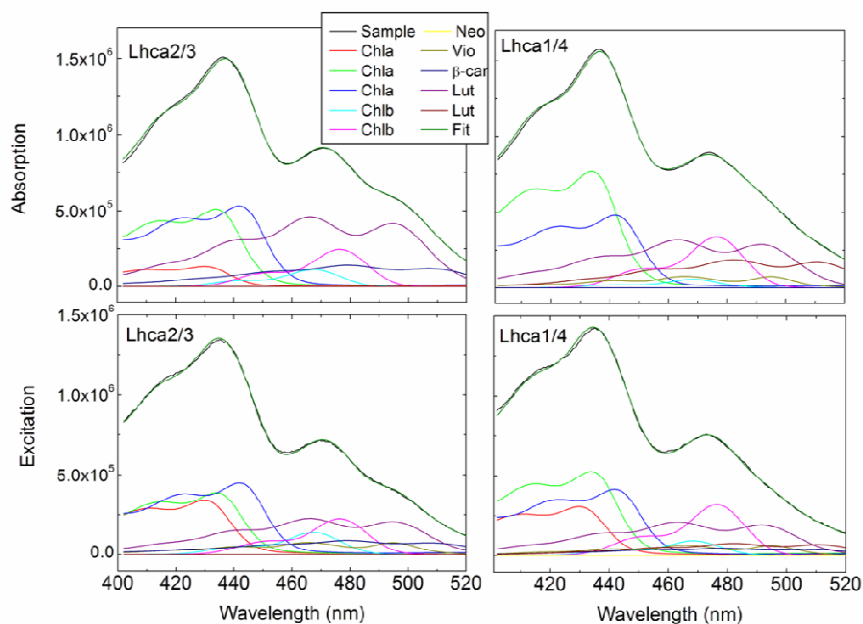


FIGURE SI2.1 Decomposition of the Soret region of the absorption (*top*) and excitation (*bottom*) spectra of the dimers in terms of the individual pigments: Chl *a*, Chl *b*, neoxanthin (Neo), violaxanthin (Vio), β -carotene (β -car) and lutein (Lut). The sum of the individual pigments is also shown (Fit).

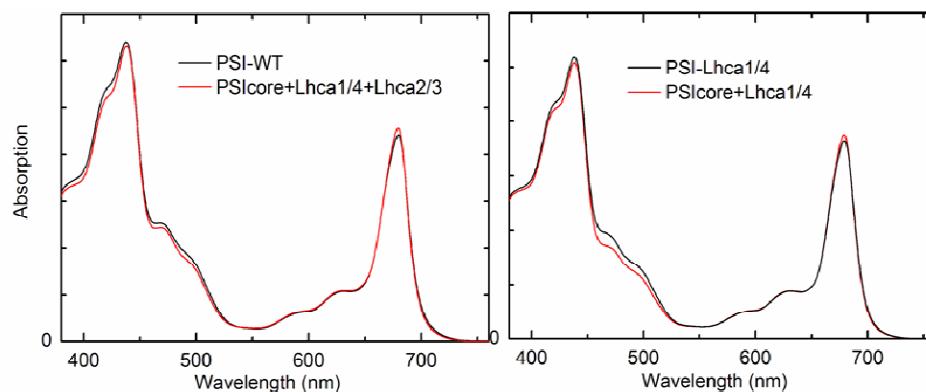


FIGURE SI2.2 Absorption spectra of PSI-Lhca complexes compared to the sum of PSI core and Lhca dimer(s).

Table SI2.1. Fraction of core excitation. Values are reported obtained with the two methods described above as Method1/Method2. For PSI-Lhca5 the absorption spectrum of Lhca1/5 is not known, thus only Method 2 is used where the LHCI Car to Chl efficiency is supposed to be the same as for WT LHCI.

	PSI-WT	PSI-Lhca1/4	PSI-Lhca5
Core excitation 440nm	0.63/0.62	0.77/0.76	/0.61
Core excitation 475nm	0.36/0.35	0.53/0.47	/0.38

SI2B Possible errors in the estimation on the core excitation fraction and the effect on LHCI to core transfer times.

To estimate the core excitation fraction a few assumptions have to be made, possibly giving rise to an error. The main uncertainties are the Car to Chl energy transfer efficiencies. The effects of assuming different transfer efficiencies for PSI-WT are reported in Table SI2.2. In column A the assumptions described in SI2A were used. In column B a Car to Chl transfer efficiency of 90% is used for the core as reported in (3). In C the absorption of the few Chls *b*, always present in the PSI core preparation of higher plants (Chl*a/b*=33), are not taken into account. This means that at 475nm only Cars are excited (which are assumed to transfer 70%), while in A 20% of Chl excitation (transferring 100%) was assumed, see Fig SI2.3. In D a higher Car to Chl transfer efficiency was assumed, comparable to values found for Lhcb complexes, e.g. (4). It should be noted that LHCI in this case also includes the gap pigments of which the transfer efficiency is unknown. In E the assumptions used in C and D are combined, these assumptions were used in van Oort et al. (5). The range of obtained $\bar{\tau}_{L-C}$ values (Table SI2.2) provides an indication of the possible inaccuracy of this value.

Table SI2.2 Core excitation fraction of PSI-WT. See text for explanation.

	A See SI2A	B 90% Car trans Core	C Chl <i>b</i> Core not considered	D 85% Car trans LHCI	E = C+D
Ex Core 440	0.62	0.63	0.62	0.60	0.62
Ex core 475	0.35	0.39	0.33	0.31	0.30
Diff	0.28	0.24	0.30	0.29	0.32
$\bar{\tau}_{L-C}$ (ps)	19.6	22.8	18.5	19.0	17.1

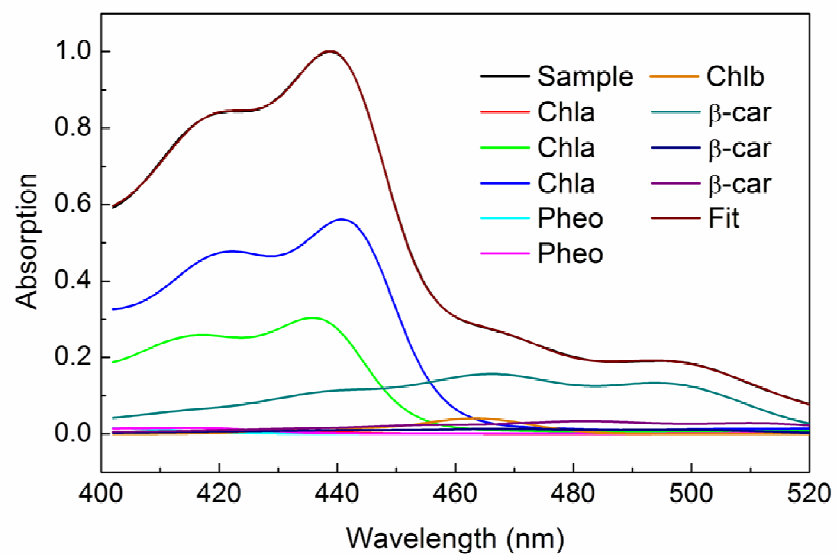


FIGURE SI2.3 Description of PSI core absorption spectrum with Chl *a*, β -carotene and a low amount of Chl *b*, furthermore a very small contribution of Pheophytin (Pheo) was needed to describe the absorption. Note that the absorption of the sample (*black*) overlaps perfectly with the sum of the individual pigments (Fit: *brown*).

SI3 PSI-Lhca1/4 is working less efficiently than the intact system

Based on the TCSPC study with two excitation wavelengths it was estimated that excitation energy transfer from LHCI to the core occurs with an average rate of 50/ns in PSI-WT, and only with a rate of 32/ns in PSI-Lhca1/4. This would thus imply about 2 times faster energy transfer from Lhca2/3 to the core, than from Lhca1/4. To investigate this in more detail the average fluorescence lifetimes of PSI-WT and PSI-Lhca1/4 are plotted as a function of the emission wavelengths (Fig SI3). After 475nm excitation PSI-Lhca1/4 has a longer average lifetime than PSI-WT for the red-shifted wavelengths. This means that the excitation energy transfer from Lhca1/4 to the core is slower in PSI-Lhca1/4 than in WT, otherwise the lifetime would always be shorter for a complex harboring less (red) Chls. Shorter lifetimes are indeed observed for PSI-Lhca1/4 after more selective core excitation (440nm) (Fig SI3). It can thus be concluded that the connectivity between Lhca1/4 and the core is better in intact PSI-WT, than in the sub-complex lacking Lhca2/3, in agreement with the LT fluorescence results (Fig 1). This also means that the difference in transfer rates from both dimers to the core in PSI-WT is smaller than a factor two, and thus that the rates are rather similar. More efficient energy transfer in intact photosynthetic supercomplexes, compared to complexes lacking some Lhc antennae, might be a general PS feature, as it has also been observed for PSII (6).

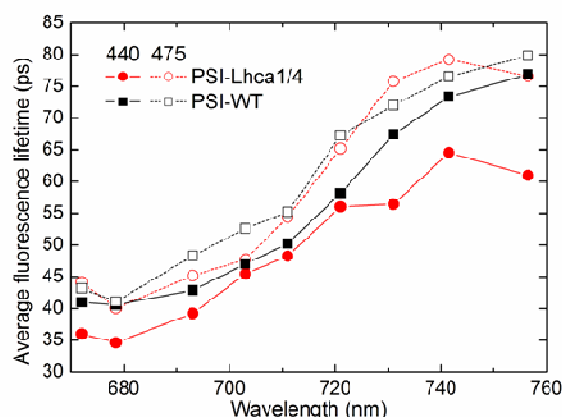


FIGURE SI3 Average fluorescence lifetime of PSI-Lhca1/4 and PSI-WT calculated from the three shortest lifetimes as found with TCSPC after 440nm and 475nm excitation (Fig 3).

SI4 Comparing the DAS from different PSI complexes.

Fig SI4 A shows that the ~80ps DAS of PSI-WT, PSI-Lhca1/4 and PSI-Lhca5 have similar red emission maxima. Fig SI4 B shows the strongly red-shifted sub-ns DAS of PSI-WT and PSI-Lhca1/4.

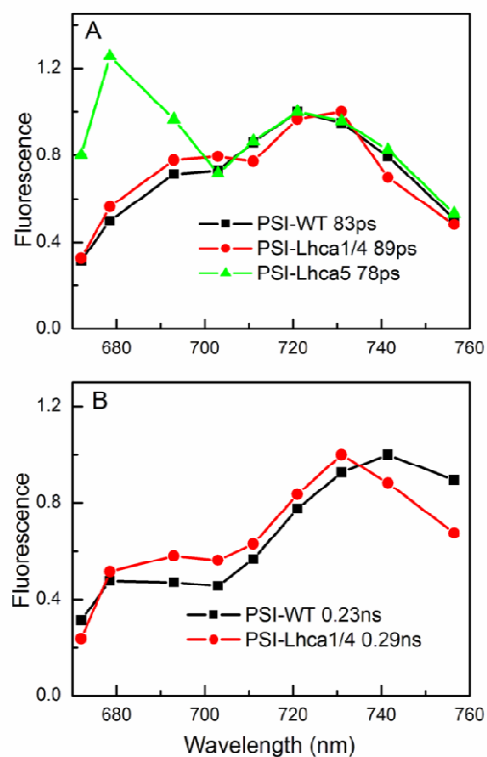


FIGURE SI4. A: decay associated spectra with ~80ps lifetime of PSI-WT, PSI-Lhca1/4 and PSI-Lhca5. B: The sub-ns DAS of PSI-WT and PSI-Lhca1/4. DAS have been normalized on the maximum of the red emission.

SI5A Determination of the Lhca to core transfer rates

In case energy equilibration within Lhca is fast compared to the energy transfer to the core, then the rates $k_{a\#C}$ (rate of energy transfer from Lhca# to core) and $k_{Ca\#}$ (rate of energy transfer from core to Lhca#) should follow the detailed balance equation. It is assumed that the core to Lhca transfer rate is equal for Lhca1 and Lhca4 (See below), thus $k_{Ca1} = k_{Ca4} = A$.

Thus for energy transfer between Lhca1 and Lhca4 and the core:

$$(1) \quad \frac{k_{Ca1}}{k_{a1C}} = \frac{n_{a1}}{n_C} * e^{-(E_{a1}-E_C)/kT} = \frac{A}{k_{a1C}} \text{ and}$$

$$(2) \quad \frac{k_{Ca4}}{k_{a4C}} = \frac{n_{a4}}{n_C} * e^{-(E_{a4}-E_C)/kT} = \frac{A}{k_{a4C}}$$

with n the number of pigments and E the excited state energy of the Lhca and the core. It follows that:

$$\frac{(Eq.1)}{(Eq.2)} = \frac{k_{a4C}}{k_{a1C}} = \frac{n_{a1}}{n_{a4}} * e^{-(E_{a1}-E_{a4})/kT} = \frac{k_{a4a1}}{k_{a1a4}}$$

where k_{a4a1} and k_{a1a4} are the rates of energy transfer from Lhca4 to Lhca1 and from Lhca1 to Lhca4, respectively. These rates have been obtained in (7).

SI5B Förster overlap integral with red forms

The Förster overlap integral J depends on the emission properties of the donor and the absorption properties of the acceptor, according to:

$$J = \int_0^\infty f_D(\lambda) * \varepsilon_A(\lambda) * \lambda^4 d\lambda$$

The spectra in Fig SI5B were used to compare the overlap integral between bulk Chl a donor and bulk Chl a acceptor, or a high red Chl a acceptor. Taking these spectra $J_{bulk-red}/J_{bulk-bulk}$ is 0.83. When the emission spectrum of the donor is 4nm red-shifted (λ_{max} 685nm) this ratio increases to 1.14. This indicates that the energy transfer from bulk Chls to bulk or red Chls are similar, under the assumption that the dipole orientation factor (κ^2) is the same.

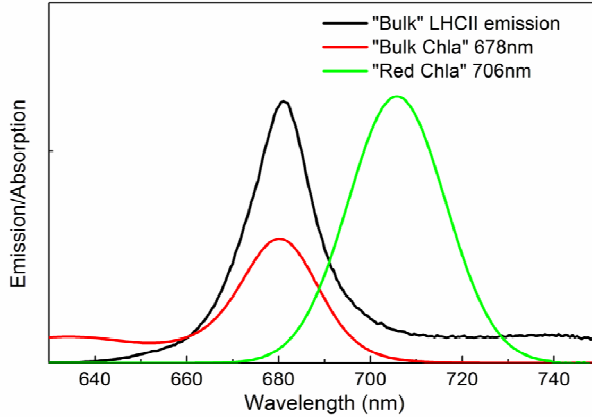


FIGURE SI5B Chl absorption and emission. Emission spectrum of LHCII (*black*), absorption band of Chl a in protein environment (8) with maximum at 678nm (*red*), Gaussian band-shape with maximum at 706nm and full width at half maximum (*green*) represents the red Chls of Lhca3 and Lhca4 (9). Oscillator strength in Q_y region is two times larger for the red Chls compared to the bulk Chl a , as was observed in (7).

SI6 Synchroscan streak camera data and target analysis description

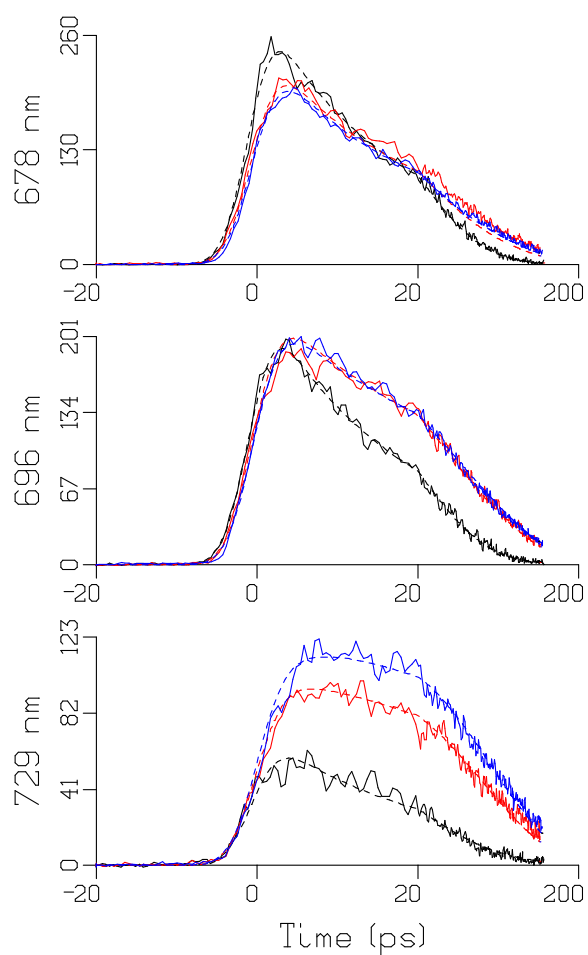


FIGURE SI6 Synchroscan streak camera decay traces (*solid*) and fit based on target analysis (*dashed*) at three detection wavelengths for PSI core (*black*), PSI-Lhca1/4 (*red*) and PSI-WT (*blue*). Note that the time axis is linear until 20 ps, and logarithmic thereafter.

SI7 Transient excitation concentration of PSI-WT compartments

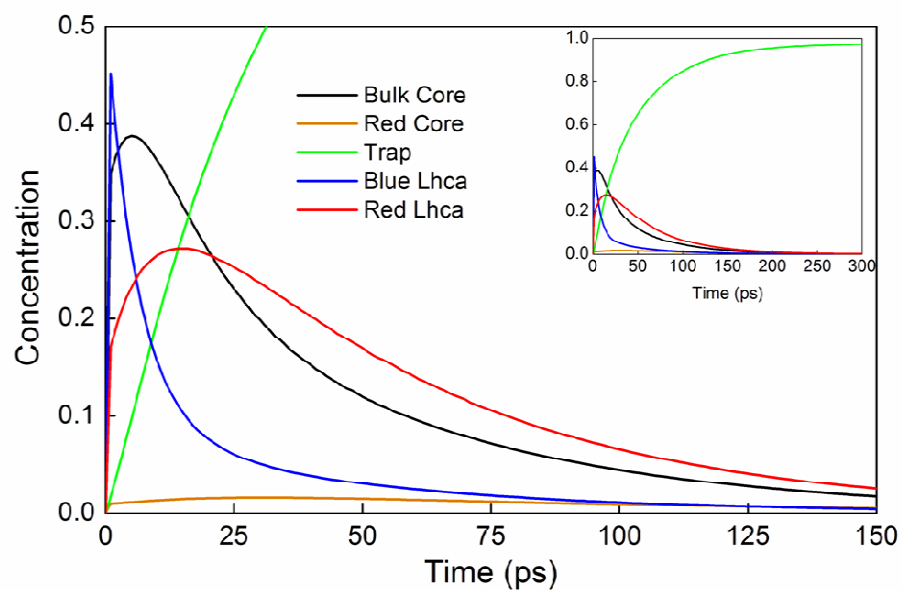


FIGURE SI7 Evolution of the excitation population of each compartment of PSI-WT.

SI8 Target analysis PSI-Lhca1/4

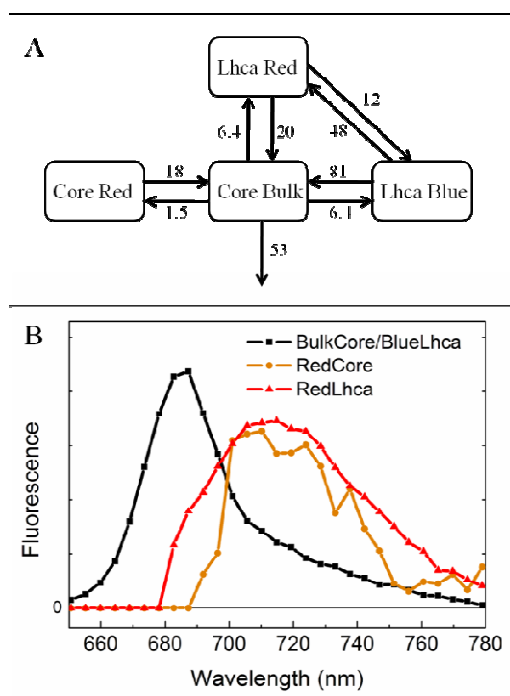


FIGURE SI8 Target analysis of PSI-Lhca1/4 kinetics. A: Compartment model of PSI-Lhca1/4, with EET rates in /ns. B: SAS of the compartments. Initial excitation fraction of the core was fixed at a value of 53% as obtained from the absorption spectra (Table 1 main text). LHCI excitation was 65%, the fractions of *red* vs *blue* antenna excitation were estimated at 0.25 and 0.75.

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